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RESEARCH PAPER

Optimization of biological hydrogen production process using stepwise regression method

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Abstract

With the aim of better understanding the influence of hydrogen partial pressure on biohydrogen production parameters, an experimental set-up was constructed of three 5.5-L continuous stirred tank reactors having three stirring speed levels (A=240, B=135 and C=80 rpm). The influence of organic loading rate (OLR), from 7.5 to 37.5 kg VS/m^3.d, on the performance of bioreactors producing fermentative hydrogen was examined. The gas amount varied according to different OLRs, but it could be stabilized at a high level, with a hydrogen concentration in the gas that ranged from 41.7–50.8% in test run A, 45-64% in test run B, and 38-50.5% in test run C. The yield in the test run B was significantly higher than both test runs A and C: 1.27 versus 0.5 and 1.05 mol-H_2/mol-glucose, respectively. At the high OLR of 30-35 kg VS/m^3.d, the H_2 yield was maximized at 1.60 and 2.08 mol H_2/mol glucose for the operation of test run B. Overall H_2 productivity was achieved to be 3.54 mmol/L×h in test run B. Stepwise regression method was then used to discover effective parameters on biohydrogen production and to achieve the best adjusting model. Experimental results showed that this method has a great capability to describe continuous hydrogen production systems. Stepwise method showed that organic loading rate, nutrient, NaOH, and hydrogen partial pressure have significant influences on hydrogen specific production rates ($p<0.002$). Adjusted coefficient R^2 increased from 0.61 to 0.71 by stepwise, entering most effective parameters into the models.

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Introduction

Ongoing fossil fuel use to meet global energy needs is causing critical environmental problems all over the world. Concerns related to these problems include energy, economic and political crises and effects on human, animal and plant health (References?). Since the oil crisis in 1973, considerable progress has been made in the search for alternative energy sources (Momirlan and Veziroglu, 2002). Modern bioenergy has received increased attention in the past decade because it not only provides an effective energy source from a technical point of view but also utilizes resources that can be sustainably obtained around the globe (Silveria, 2005).

Hydrogen, an entirely carbon-free fuel with a high combustion enthalpy (141.9 kJ/g), is considered a feasible alternative to fossil fuels, and the technology for using hydrogen as a transport fuel is already well established. Hydrogen is a promising, ideal fuel of the future that offers many social, economic and environmental benefits. In the long term, it has the potential to reduce the global dependence on imported oil and also to decrease carbon and other emissions from the transportation sector. The idea of a post-fossil fuel, hydrogen-based economy began to gain mainstream interest only in the last decade (Kotay and Das, 2007).

Hydrogen is currently produced in large amounts in the chemical industry, including steam reformation of fossil fuels. Hydrogen must be produced sustainably to be economically feasible. This could be achieved from water through electrolysis powered by photosynthetic or other renewable energy, or by gasification or pyrolysis of biomass. It also may be possible to develop a cost-effective and reliable technology to produce hydrogen directly from renewable biomass or organic waste products through anaerobic fermentation (Hawkes *et al.,* 2002). Strategies for H_2 production from plant sources essentially follow two major routes: photochemical conversion of sunlight and dark fermentative processes. Although many scientific issues still require research, the fermentative path currently appears to be closer to practical utilization. The benefits of this approach include the low cost of biomass and the fact that the byproducts of agricultural food production can be used as feedstock (Fan *et al.,* 2006, Pattra *et al.,* 2008 and Kayazze *et al.,* 2008).

Because of Iran's rich fossil energy resources, very little attention has been paid to other production parts and majority of the country's financial income is supplied by pressure on the natural resources. Approximately 17.86 MT of crop waste is produced in Iran, which can potentially produce 4.91 GL of bioethanol in a year. Wheat, sugar cane bagasse, rice, barely and corn are the most favorable biofuel production sources in Iran (Najafi *et al.,* 2009). Only small quantities of these were used to make paper or feedstuffs for livestock. The rest is mostly burnt or discarded causing environmental pollution.

Hydrogen production from different smaller volume biomass materials such as beer less, cornstalk, wheat straw, dried distillers grain and barley hulls (Mangnusson *et al.,* 2008, Chen *et al.,* 2009, Zhang *et al.,* 2007, and Nasirian *et al.,* 2011) or carbohydrates or starch (Oh *et al.,* 2004; Ren *et al.,* 2006; Yu and Mu 2009; Lin *et al.,* 2006; Ueno *et al.,* 2009; Roychowdhury *et al.,* 1988) using different microbes such as *Enterobacter aerogenes* (Rachman *et al.,* 1997), *Clostridium* sp (Skonieczny and Yargeau, 2009) and *Aspergillus terreus* (Emtiazi *et al.,* 2001) or mixed cultures (Chen *et al.,* 2009 and Nasirian et *et al.,* 2011) in different reactor configurations like continuous (Hawkes *et al.,* 2002), membrane bioreactor (Oh *et al.,* 2004; Ren *et al.,* 2006) and UASB reactor (Yu and Mu, 2006, Lin *et al.,* 2006) has been reported in literature. It is noticed that, the H_2 yield is influenced by the type of material, its pre-processing and fermentation conditions (Chen *et al.,* 2009; Zhang *et al.,* 2007), which emphasizes the need for optimization. Statistical methodologies offer enormous advantage over conventional methods in process optimization (Yang *et al.,* 2007; Sreenivas, 2004, Sreenivas *et al.,* 2004; Sreenivas *et al.,* 2008; Prakasham *et al.,* 2005; Suba *et al.,* 2008; Prakasham et al., 2009; Obeid *et al.,* 2009; Prakasham *et al.,* 2006; Prakasham *et al.,* 2007; Sathish *et al.,* 2008). Only a few reports in literature are available on the optimization of biohydrogen yield (Gustavo *et al.,* 2008; Sompong *et al.,* 2008; Mu *et al.,* 2006; Wang *et al.,* 2009). Among different statistical optimization methodologies, stepwise regression is a special class of regression analysis where many variable factors considered important in product design, and/or in development activities for industrial applications involving formulations or mixtures, may be analyzed.

Based on this background, a focus has been made to explore the feasibility of waste sugar as substrate source for biohydrogen production using stepwise regression, and to verify how the H_2 production yields are affected by variation of proportions of the process conditions.

Materials and methods

Hydrogen-producing microflora and pretreatment

The seed sludge of mixed culture for the initial experimental runs was obtained from an anaerobic digester in Duisburg Municipal wastewater treatment plant in Duisburg-Essen, Germany, and heat treated at 70 °C for 60 minutes (min) to inhibit methane-producing bacteria (Nasirian et al., 2011). For next trials the seed sludge of mixed culture was obtained from a continuously stirring tank reactor that was producing hydrogen by fermenting waste sugar at 36 ±1 °C and pH 5.2-5.4. The reactor had been operating continuously for two months and produced a biogas composed of more than 60% hydrogen.

Experimental procedure

The continuous tests took place under mesophilic conditions in 5.5-L reactors (Fig. 1). The reactors were made of acrylic glass and had a working volume of 4.5 L. In three experiments, following a batch start-up (24h to 48h), the systems were converted to operate continuously. The reactors were operated at pH 5.2, 36 °C and with hydraulic retention times (HRT) of 32, 24, 20, 16, 14, 12, 10 or 8 hours (h). Details of the experiments are shown in Table 1. Experiment A continued for 46 days, Experiment B for 110 days and Experiment C for 35 days. Reactors were stirred using an adjustable stirrer at 80, 135 and 240 rpm. The temperatures in the reactors were set to 35 °C to 37 °C by chemostats (Thermo, Karlsruhe, Germany and Julabo, Seelbach, Germany) through a water-jacket, as is common in anaerobic tests (DIN 38414 S8). The pH value was kept constantly between 5.2 and 5.4 by automatic titration using peristaltic pump connected to an IKS controlling computer (IKS Computer Systems, GmbH, Karlsbad, Germany), using 1 M NaOH. The liquid volume of each reactor was recorded and adjusted by filling and emptying if necessary. The reactors were loaded and emptied using Heidolph (Schwabach, Germany) tube pumps. The seed sludge was acclimated with waste sugar as carbon source.

To improve the fermentation conditions, a nutrient was added to each system. Mineral nutrients were added at the following concentrations (modified based on Hussy et al. (Hussy et al., 2003)): NH_4Cl 2600 mg, K_2HPO_4 250 mg, KH_2PO_4 250 mg, $MgCl_2$. $6H_2O$ 320 mg, $FeSO_4$. $7H_2O$ 86 mg, $CoCl_2$. $6H_2O$ 15 mg, $MnCl_2$. $4H_2O$ 15 mg, (NH_4) $6Mo_7O_{24}$. $4H_2O$ 14 mg, $Na_2B_4O_7$. $10H_2O$ 12 mg, $NiCl_2$. $6H_2O$ 49 mg, $ZnCl_2$ 23 mg, $CuCl_2$. $2H_2O$ 10 mg and $CaCl_2$. $2H_2O$ 66 mg. The solution was stored as a 13-fold concentrate and acidified to pH < 2. During all tests, a nutrient dilution was added to media to ensure a proper supply of trace elements, nitrogen and phosphorous. During all test runs the feeding intervals were adapted to the designated HRT and controlled by computer fitted with Labview software controller system. The culture's pH, reactor temperature and air pressure were monitored continuously. Data was logged every 5 minutes to a Pentium IV computer fitted with a Labview data acquisition system. The effluent gas flow rate was measured using a volumetric low flow gas meter (Milligas counter, Dr. Ritter Gasapparatebau, Bochum, Germany).

Calculated parameters

The daily volume of produced gas of each trial was estimated and converted to standard volume (0 °C, 1013 hPa). The degree of degradation ($\eta_{C,\ gas}$) was calculated by dividing the carbon weight from fermented gas (produced in CO_2 form) by the carbon weight of the input substrate to demonstrate the degradability of input material to fermentation gas. The degree of degradation was determined based on the CO_2 content of the gas phase (C_{gas}). Equation 1 shows the calculation of the degree of degradation (Krupp and Widmann, 2009).

$$\eta_{C,\, gas} = (C_{gas} / C_{solid,\, input}) \times 100 \quad (1)$$

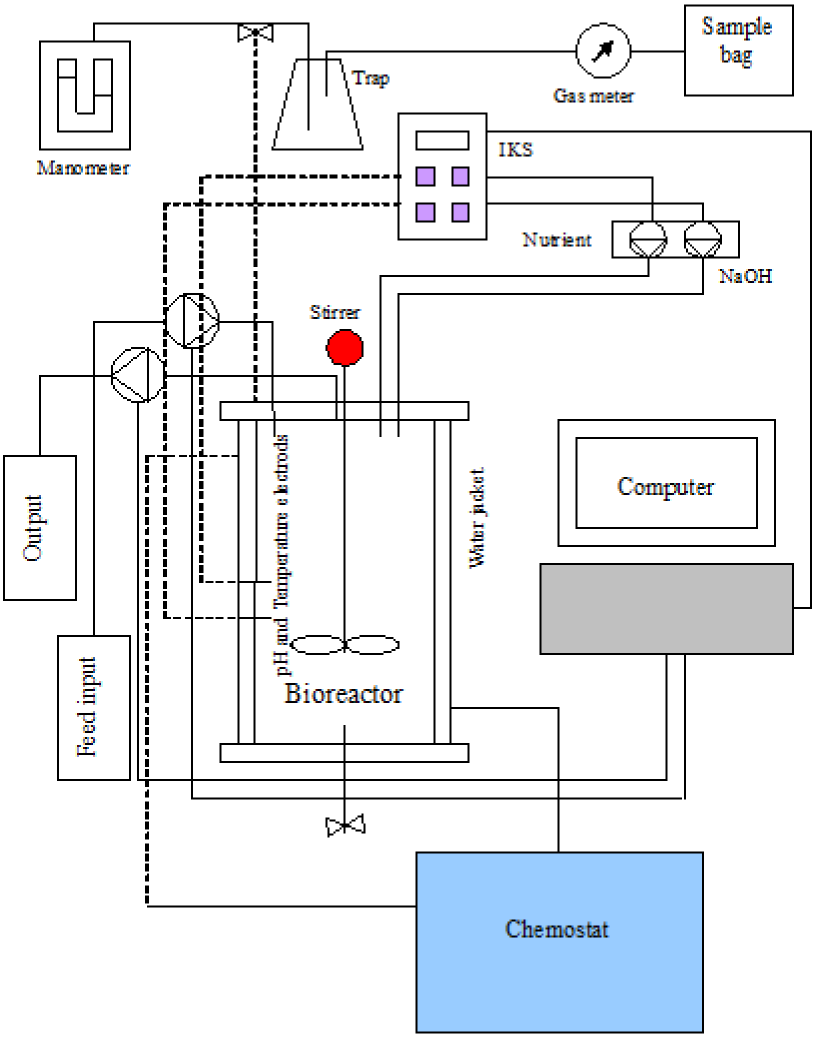

Fig. 1. Schematic of the continuous biohydrogen production system.

Analytical methods

The produced gas was collected in special bags, and its volume was measured by low-flow gas meter. The volumes of gas produced daily in each trial were converted to standard volumes. The hydrogen content of the produced gas was determined off-line daily with a Conthos 2 process gas analyzer based on thermal conductivity (LFE, Maintal, Germany). Amounts of CO_2, CH_4 and O_2 were measured off-line daily with a GA94 infrared gas analyzer (Geotechnical Instruments/Ansyco, Karlsruhe, Germany). The amounts of organic acid were also measured daily as equivalents of acetic acid by the Hach-Lange cuvette test LCK 365 (50-2500 mg/L). Chemical Oxygen Demand (COD) was determined photometrically using Hach-Lange cuvette tests according to DIN 38409 H41. COD was determined photometrically by measuring the color change of a potassium dichromate dilution with an MDA photometer ISIS 9000, type LPG 282 (Hach-Lange Dusseldorf, Germany). Total Solids (TS) and Volatile Solids (VS) of the sludge inocula and input and output test samples were determined by standard methods DIN38414 S2 and S3. Carbon and nitrogen were determined with a vireo MAX CN analyzer (Elementar Analysis Systems, Hanau, Germany). Dissolved Organic Carbon (DOC) levels were determined with a DIMATOC 2000 DOC analyzer (DIMATEC Analysentechnik GmbH, Essen, Germany). To obtain Total Organic Carbon (TOC), the samples were filtered using a <45-µm celluloseacetate filter (Whatman, Brentford, UK) before analysis.

Stepwise regression model

Different types of multiple regression are distinguished by the method for entering the independent variables into the analysis. In standard (or simultaneous) multiple regression, all of the independent variables are inserted into the analysis at the same. In hierarchical (or sequential) multiple regression, the independent variables are inserted in an order prescribed by the analyst.

In stepwise (or statistical) multiple regression, the independent variables are inserted according to their statistical contribution in explaining the variance in the dependent variable. Stepwise regression is designed to find the most parsimonious set of predictors that are most effective in predicting the dependent variable. Variables are added to the regression equation one at a time, using the statistical criterion of maximizing the R^2 of the included variables. The process of adding more variables stops when all of the available variables have been included or when it is not possible to make a statistically significant improvement in R^2 using any of the variables not yet included. These variables are removed if their p-values are greater than the Alpha to enter value. Since variables will not be added to the regression equation unless they make a statistically significant addition to the analysis, all of the independent variable selected for inclusion will have a statistically significant relationship to the dependent variable.

Results and discussion

The most important information provided by the continuous tests is the amount of produced biogas and its composition, which allows identification of the best method for preparing the substrate for degradation by the biohydrogen-producing bacteria. Three experiments were performed with different process conditions and were compared by the parameters of biohydrogen production. To make the methods comparable, the results were first standardized by determining the hydrogen production efficiency and calculating the degree of degradation based on carbon input and dioxide output.

Process conditions of continuous hydrogen producing systems

As described in the previous section, all the three reactors were stirred to stabilize in different specific speeds. To achieve the highest hydrogen yield, hydrogen loading rate was changed by varying hydraulic retention time of the substance or the initial concentration. In all different rates, the system was allowed enough time to reach the stable condition. Working conditions of each reactor is demonstrated in Table 1.

Table 1. Processes conditions and their corresponding working time.

Run	Duration of cont. Feeding (days)	HRT (h)	C (kg/m^3)	Stirring Speed (RPM)
A	46	32, 24, 16, 12	10	240
B	120	32,24,16,14,12, 10,8	10 & 12	135
C	35	32,24,20,16,12	10	80

Table 2. Average main Operational parameters in reactors.

Parameters	Reactor name	Mean	Std. deviation	Min	Max
HC (%)	A	47.27	1.79	43.70	50.80
	B	57.60	4.28	45	64
	C	45.36	3.05	38	50.50
HSPR (mmol/L.h)	A	0.89	0.38	0.29	1.49
	B	1.47	0.74	0.16	3.54
	C	0.79	0.27	0.15	1.29
HY (mol H_2/mol glucose)	A	0.50	0.33	0.14	1.24
	B	1.27	0.63	0.20	2.83
	C	1.05	0.52	0.11	2.56
$\eta_{C, gas}$ (%)	A	11.75	7.42	3.37	27.26
	B	8.96	4.87	1.60	32.11
	C	13.14	6.44	0.39	22.31
VFA (g/d)	A	11.07	2.92	5.27	16.79
	B	14.53	7.57	1.68	35.30
	C	14.53	2.67	9.32	19.94
H_2/CO_2 (-)	A	0.90	0.08	0.81	1.03
	B	1.36	0.20	0.86	1.78
	C	0.98	0.07	0.88	1.16

Table 3. Descriptive statistics.

	Mean	**Std. Deviation**	**N**
NSHPR (NL/Lmedia.d)	0.68	0.41	120
NaOH (mL/d)	132.60	55.16	120
Nutrient (mL.d)	11.95	8.07	120
T (°C)	35.50	0.75	120
P_{ab} (hPa)	1006.00	10.10	120
Real OLR (gDOC/L.d)	8.09	4.49	120
pH	5.32	0.11	120

One notable point is that hydrogen yield in A and C reactors are stopped after decreasing the HRT to 12 hours. This could be a result of microorganisms' deconstruction and preventing the system from reaching a stable condition.

Effects of different stirring speeds and organic loading rate on bio-hydrogen production parameters

Table 2 summarizes experimental data obtained at each stirring speed and various OLRs (7.5~37.5 g VS /L.d) for the reactors that reached steady state conditions. The gas amount varied with the different OLRs, but could be stabilized at a high level, with the hydrogen concentration in the gas ranging from 41.7–50.8% in test run A, 45-64% in test run B and 38-50.5% in test run C. The hydrogen gas productivity and concentration were higher at 135 rpm than other rates with a HRT decrease from 32 to 8 h. Overall H_2 productivity was achieved to be 3.54 mmol/L×h in the test run B, and the mean value was 1.47 mmol/L×h over the 125 days of test run B operation versus 0.89 and 0.79 mmol/L×h in test runs A and C, respectively.

Table 4. Equation coefficients and meaningfulness of the model's variables.

Predictor Variable	**Beta**	**P <**
Real OLR (gDOC/L.d)	0.04	0.000
nutrient (mL/d)	0.013	0.021
NaOH (mL/d)	0.002	0.000
Pab (hPa)	0.006	0.001

The average H_2/CO_2 was more than 1.2 in 135 rpm during 65 days test operation by decreasing OLR from 17.14 to 37.5 g VS/L.d. It was achieved to high level of performance up to 1.8 in 30 g VS/L.d. During days 74-125, H_2 content was stabilized on more than 60% in reactor B with a HRT decreased from 12h. With HRT decreasing from 16 to 12h, clear signs of washout appeared at 80 rpm stirring speed. In addition, at 80 rpm as well as 240 rpm the gas production from the reactors decreased gradually and ceased on day 33 and 45 respectively.

The best hydrogen yield (2.83 mol H_2/mol glucose) was found in reactor B with an HRT of 8h and an OLR of 37.5 g VS/L.d. The average yield in the test run B was significantly higher than that in the test runs A and C, 1.27 versus 0.5 and 1.05 mol-H_2/mol-glucose, respectively. At the high OLR of 30-35 kg VS/m^3.d the H_2 yield was maximized at 1.60 and 2.08 mol-H_2/mol-glucose for the operation of test run B. These results suggest that an optimum hydrogen partial pressure that maximizes H_2 yield and H_2 production could be achieved on test run B with 135 RPM.

Results show that to achieve a best and most stable production condition, we need to optimize reactor function. To do this, it is essential to produce an accurate model to describe system working conditions, the effective parameters and as a consequence, controlling system performance in an optimal production mode. Considering the described properties of the stepwise regression model, we decided to study reactor working conditions and the effective production parameters.

Bio-hydrogen production: Modeling and effective parameters

Essential assumptions to perform a stepwise regression analysis

One hundred and twenty (120) observations from the best test reactor were recorded. This data set contained more than 5 times the number of independent variables required, and produced the required initial conditions of the stepwise regression test. Independent variables are studied in terms of multicollinearity and were discovered not to have strong dependence. By studying residual distribution tables and the forecasted values it was observed that there exists no vivid relationship between the residual and the predicted values, which is compatible with the initial assumption that there is a linear relationship. It is also observed that for the dependent values, the residuals are normally distributed. That is because according to this Figure, if all points are on the bisector, the results are having a normal distribution.

Regression model

Considering the achieved results and the initial tests on observed data, calculations were done using stepwise regression method in Minitab 16 software. Descriptive statistics and dependent and independent variables' properties are seen in Table 3.

Four regression models were achieved to increase the multiplexed, determined, adjusted coefficient R^2 from 0.61 to 0.71. All adjusted regression models were totally meaningful in a probability level of 1%. Independent variables were also studied, according to which, it is proved that static variables of the model have had meaningful effect on the hydrogen production forecast.

Adjusted $R^2 = 0.71$; $F_{4,170} = 70.29$, $p < 0.000$ (using the stepwise method).

Significant variables are shown below:

$$\text{HSPR} = -6.28 + 0.04\ \text{OLR} + 0.013\ \text{nutrient} + 0.002\ \text{NaOH} + 0.006\ P_{ab}$$

As observed, the key parameters that have the greatest effect on optimizing hydrogen production include organic loading rate, NaOH, and hydrogen partial pressure. In this section, after doing a regression analysis, OLR's share in describing optimized hydrogen production variance was set to 40% and in the next step from the values of R square change and Significant F change, we realized that nutrient has had 13% meaningfulness for hydrogen production optimization variance. Therefore, considering the above equation, it is possible to describe and optimize reactors' working conditions. The reactor A and C were stopped on different days, because of microorganism washout, however, the model was forecasted hydrogen productivity of the reactors with different performances accurately.

Conclusions

The present work focused on optimization of key parameters for improving the specific hydrogen production rate using a statistical methodology. Results suggest that reactor B, having a stirring speed of 135 rpm and in an OLR between 30 and 35 $kg_{VS}/m^3.d$ produce the best result. Overall H_2 productivity was achieved to 3.54 mmol/L×h in the test run B, and the mean value was 1.47 mmol/L×h during 125 days of operation. In addition, the best hydrogen yield was 2.83 mol H_2/mol glucose on 8h of HRT and 37.5 g VS/L.d of correspondence OLR. A Stepwise Regression method was then used to elucidate the effective parameters on biohydrogen production and to achieve the best adjusting model. Stepwise method showed that organic loading rate, nutrient, NaOH, and hydrogen partial pressure had significant influences on hydrogen specific production rates ($p<0.002$). Adjusted coefficient R^2 increased from 0.61 to 0.71 by stepwise entering most effective parameters into the models. Although the reactor performances were different, the provided model showed great capability to predict continuous hydrogen production under various process conditions.

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